

What is claimed is:

*A1*  
*Sub* 1. A method for producing hemoglobin solution from a solution containing red blood cells within an automated blood separator comprising a centrifuge, the method comprising the steps of:

- 5 (a) isolating the red blood cells in the solution;  
(b) removing the supernatant produced during the step of isolating;  
washing the red blood cells in a wash solution;  
(c) lysing the red blood cells to produce a hemolysate; and  
(d) separating stromata of the red blood cells from the hemolysate.

10 2. The method of claim 1, wherein the wash solution comprises a normal saline solution.

3. The method of claim 1, wherein the wash solution comprises an agent for killing bacteria.

15 4. The method of claim 1, wherein the wash solution comprises an agent to inactivate viruses.

5. The method of claim 1, wherein steps (a) through (d) are performed within a single processing container disposed within the centrifuge.

20 6. ~~The method of claim 1, further comprising the step of measuring an ionic concentration of the hemolysate and wherein steps (c) and (d) are repeated until the ionic concentration decreases to a predetermine level.~~

*SUB A2* 7. A method performed within a cell processing apparatus for producing hemoglobin from a solution containing red blood cells and plasma, the method comprising the steps of:

25 collecting the solution in a sterile processing set comprising a processing bag and a tube harness, wherein the processing bag is disposed within a centrifuge in the cell processing apparatus;

separating the red blood cells from the plasma by rotating the processing bag within the centrifuge;

expressing the plasma from the processing bag;

30 introducing a washing solution into the processing bag to wash the red blood cells;

expressing the supernatant after washing;

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lysing the red blood cells by introducing distilled water into the processing bag to liberate hemoglobin in solution;

separating red blood cell membranes from the hemoglobin solution by rotating the processing bag in the centrifuge; and

5 removing the hemoglobin solution through a sterile port in the processing bag.

8. The method of claim 7, wherein the step of separating red blood cell membranes from hemoglobin comprises the steps of:

10 removing the first-produced hemoglobin produced when the distilled water initially contacts the red blood cells; and

continually removing additional hemoglobin produced as an ionic strength of the solution decreases.

9. The method of claim 8, further comprising the steps of :

15 measuring the ionic strength of a hemoglobin solution produced by the step of removing the hemoglobin from the solution;

adding additional distilled water; and

repeating the steps of removing the additional hemoglobin and addition the additional distilled water until the ionic strength reaches a predetermined threshold.

20 10. A method of separating hemoglobin from packed red blood cells within a processing container in a cell processing apparatus, the method comprising the steps performed within the apparatus of:

washing the red blood cells in the processing container with a saline solution;

lysing the red blood cells in the processing container;

25 separating red blood cell membranes and unlysed red blood cells from hemoglobin within the processing container; and

extracting the hemoglobin in solution from the processing container.

30 11. The method of claim 10, wherein the step of separating comprises centrifuging the processing container within the apparatus to pack the red blood cell membranes and unlysed red blood cells.

12. The method of claim 10, wherein the step of washing includes adding a detergent, antibacterial or antiviral agent to the saline solution.

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13. ~~The method of claim 10, further comprising the step of measuring an ionic strength of the hemoglobin solution, wherein the steps of lysing, separating and extracting are performed repeatedly until the ionic strength decreases to a predetermined level.~~

SUB A3 5 > 14. A method for preparing a modified hemoglobin solution comprising the steps of:

mixing packed red blood cells into a normal saline solution;

washing the red blood cells with a saline solution;

lysing the red blood cells;

10 diafiltering the red blood cells to remove the stromata;

diafiltering the filtered red blood cells;

collecting the resulting hemoglobin solution in a container, wherein the container contains premeasured reagents adapted for chemical modification of the hemoglobin solution;

15 reacting the hemoglobin solution with the reagents; and

storing the reacted solution in a storage container.

~~15. The method of claim 14, wherein the premeasured reagents comprise buffer salts.~~

SUB A4 > 20 16. The method of claim 15, wherein the buffer salts are iminothiolane and activated polyethylene glycol.

17. The method of claim 14, further comprising filtering the reacted solution.

18. The method of claim 14, further comprising sterilizing the reacted solution to remove or inactivate organisms.